

The lymphocyte transformation test for the diagnosis of Lyme borreliosis has currently not been shown to be clinically useful

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This letter is a comment on a study using the lymphocyte transformation test (LTT) for the diagnosis of active Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato [1]. This LTT study reports the findings derived from a validation panel containing 120 blood donors seronegative for *Borrelia*, 40 seronegative patients with autoimmune diseases, 48 healthy seropositive controls, and 94 seropositive patients with clinical signs of Lyme borreliosis. Furthermore, 1480 samples

were investigated with both serology (*Borrelia* IgG and IgM ELISA, and western blot; Mikrogen, Munich, Germany) and the LTT.

The study has several major shortcomings. Concerning inclusion criteria, it was not clearly specified how the 94 patients with clinical Lyme borreliosis were defined. For example, it was not specified whether the six patients with Bannwarth's syndrome had spinal pleocytosis and a positive antibody index, as required by the European case definitions for Lyme borreliosis, and it remains unclear how it was determined that the 34 patients with migratory arthralgias were suffering from Lyme borreliosis [2]. The 160 controls for the LTT were preselected as being seronegative for *Borrelia*-specific antibodies, and this could introduce a selection bias, because serology and LTT results tend to correlate. The specificity of the LTT could therefore be overestimated. Concerning the selection criteria for the large group of 1480 patients, it is not clear what is meant by 'clinical diagnosis of suspected Lyme borreliosis', among what appears to be a mixture of protean disorders. The clinical spectrum of these patients was not described. Concerning the methods, it is confusing to the reader that a cut-off for a positive stimulation index may be both >5 and >3 . In the results section, the selection of subsets in the tables numbered 2–5 was not explained, and the numbers do not add up. For example, 592 of the 1480 patients were reported to be LTT-positive; however, only 340 reappear in Table 3, without an explanation of how this subset was selected. A flow diagram would have been helpful. Forty per cent of the 1480 patients suspected of having Lyme borreliosis were LTT-positive, and 63% were serology-positive. This is a high percentage of positive results as compared with a series of consecutive patients suspected of having Lyme borreliosis in Denmark, where 9.2% were found to be IgM-positive and 3.3% IgG-positive. This indicates either selection bias or specificity problems in the LTT and/or the serology assay.

The main point of the article as taken from the title is the ability of the LTT to detect active infection and the effect of antibiotic treatment. However, owing to the study design, evidence of active infection is lacking. Clinical features, including follow-up and/or detection of the organism by culture or PCR, are absent. Also, the conclusion that the *Borrelia* LTT may be used for follow-up monitoring of disseminated *B. burgdorferi* sensu lato infections and provide indications for antibiotic treatment is not supported by the study design, as this would require a prospective trial with a control group. Thus, the LTT paper contains methodological shortcomings with a risk of selection bias, and the study design and the data do not support the content of the title or the

conclusions about the diagnosis of active infection or measurement of treatment effect. There are several issues that need clarification in order to allow valid conclusions to be drawn about the *Borrelia* LTT.

An ethics statement is missing, and the authors declare no conflict of interest, but are associated with a commercial laboratory recommending this test (<http://www.imd-berlin.de/leistungsschwerpunkte/borreliose/ltt-borrelien.html>). This website indicates that a positive LTT result may indicate a persisting infection. However, both neurological and microbiological European guidelines discourage the use of LTTs, owing to insufficiently rigorous validation and a low reported specificity [2–4].

The development of a biomarker for active infection with *B. burgdorferi* sensu lato would be of clinical value, as antibody detection cannot currently distinguish active infection from immunological memory resulting from past or asymptomatic infection. However, T-cell recognition may be inherently indiscriminate, and problems with specificity may therefore be hard to avoid. A recent Swedish study did not find the ELISPOT technique to be useful for supplementary clinical diagnosis, with a specificity of just 82%.

The LTT study was part of a special issue entitled 'Chronic or Late Lyme Neuroborreliosis: Present and Future' in the *Open Neurology Journal* (vol. 6, 2012). However, 'chronic Lyme borreliosis' is a problematic concept, as discussed elsewhere [2,5]. As an example, it is stated that 'over 250 peer-reviewed scientific articles demonstrate the causal association between Lyme/tick-borne disease and mental illness'. This is contradicted by the conclusion based on a substantial review of the literature (Final Report of the Lyme Disease Review Panel of the Infectious Diseases Society of America; www.idsociety.org). In the Infectious Diseases Society of America review, it was determined that the large number of scientific articles concerning 'chronic Lyme borreliosis' were uncontrolled case observations, which do not give convincing evidence of the persistence of viable organisms or the effects of prolonged antibiotic treatment. In our opinion, a causal association is not demonstrated by this type of study.

The *Bentham Open Neurology Journal* is indexed in several databases such as PubMed by the National Library of Medicine and EMBASE. According to the journal website, peer review is performed. However, the issues discussed above should have been addressed by the peer review process prior to publication. It is apparent that open-access journals may have problems with the quality of peer review, and concerns about this issue have been raised elsewhere (e.g. in *Science*).

In conclusion, the clinical value of the LTT for the diagnosis of active Lyme borreliosis was not supported the von Baehr *et al.* study [1]. Critical reading of the scientific literature is necessary, with special attention to adequate standards of peer review in the increasing number of open-access journals.

Transparency Declaration

All authors contributed to the conception and intellectual contents of this commentary. R. Dessau wrote the drafts. R. Dessau declares a conflict of interest outside the submitted work. The other authors declare no conflicts of interest. For the preparation of this letter, a total of 20 references were considered, and the list may be obtained by contacting the corresponding author.

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